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Report to the Mobile Bay Enforcement Conference on Bacteriological Criteria for Shellfish Harvesting

Mobile Bay Technical Committee, Ala

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A Conference on the Matter of Pollution Affecting Shellfish in Mobile Bay, Alabama was held in the city of Mobile on January 27-28, 1970. The Conference Proceedings contained a recommendation that emphasized the need for reevaluation of the bacteriological criterion for shellfish harvesting areas. This is described in the report.

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REPORT TO THE
MOBILE BAY ENFORCEMENT CONFERENCE
ON
BACTERIOLOGICAL CRITERIA FOR SHELLFISH HARVESTING

by

MOBILE BAY TECHNICAL COMMITTEE
MOBILE BAY, ALABAMA

AUGUST 1972

Revised October 5, 1972

MOBILE BAY TECHNICAL COMMITTEE

Member	Representing
Robert F. Schneider, Chairman	Environmental Protection Agency, National Field Investigations Center-Denver, Colorado.
W. F. Anderson	Alabama Department of Conservation and Natural Resources.
Arthur N. Beck	Alabama Water Improvement Commission and Alabama State Health Department.
J. David Clem	Department of Health, Education and Welfare, Public Health Service, Food and Drug Administration.
James H. Coil, Jr.	Scott Paper Company, Mobile, Alabama.
C. B. Kelly	Environmental Protection Agency, Water Supply Programs Division.
F. James Silva	Environmental Protection Agency Region IV, Atlanta, Georgia.

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INTRODUCTION

A Conference on the Matter of Pollution Affecting Shellfish in Mobile Bay, Alabama was called by the Secretary of the Interior, and was held in the City of Mobile on January 27-28, 1970. The Conference Proceedings[1/] contained a recommendation (No. 6) that emphasized the need for reevaluation of the bacteriological criterion for shellfish harvesting areas. The recommendation read: "In determining acceptability of areas in Mobile Bay for shellfish harvesting, a more realistic criterion than the present method be employed to more effectively evaluate the probability of pathogen presence. A coordinated effort by the U. S. Department of Health, Education and Welfare and the U. S. Department of the Interior, in cooperation with the States, should be undertaken immediately to develop such a criterion with respect to approval of areas for shellfish harvesting. A technical committee shall be established by the Conferees to assist in the development of such a criterion. This committee should report to the Conferees no later than one year from the date of the first session of conference."

After a lengthy delay, the Conference summary was approved; on November 30, 1971 the EPA Enforcement Division, Region IV, Atlanta, Georgia requested that the National Field Investigations Center-Denver establish a technical committee in compliance with the Mobile Bay

Enforcement Conference Recommendation No. 6. Subsequently, through the Conferees the Technical Committee membership was established in February, 1972. (Appendices A and B)

This report summarizes the considerations used by the Mobile Bay Technical Committee for proposing a substitute bacteriological criterion to evaluate the probability of pathogen presence in Mobile Bay shellfish harvest areas.

HISTORICAL REVIEW OF THE COLIFORM BACTERIA CRITERION

The Mobile Bay Technical Committee reviewed the historical and current rationale behind the present bacteriological criterion for shellfish growing areas.

Near the turn of the century, investigators found that Escherichia coli and other related bacteria normally occurred in the intestinal tracks of mammals.[2] This assemblage of intestinal organisms became known as the coliform bacteria group. Later, it was discovered that the coliform bacteria were consistently present in sewage, polluted water, and contaminated soil,[3] [4] [5] and the coliform organisms became accepted as indicators of fecal pollution. Furthermore, it was found that water containing coliform bacteria often contained enteric pathogens; thus the results of the "coliform test" have become important considerations in judging the sanitary quality of water in shellfish harvesting areas.

In 1936[6], Dr. C. A. Perry proposed that Escherichia coli rather than other coliform group bacteria be the index of pollution for both

shellfish and shellfish waters. Five years later, a report titled, "Public Health Aspects of Clamming in Raritan Bay" concluded that "70 coliforms/100 ml, on the basis of average results from waters overlying the shellfish beds, is recommended as the limiting allowable standards for the taking of hard clams to be eaten raw."

The 1946 Manual of Recommended Practice for Sanitary Control of the Shellfish Industry[7] presented the bacteriological criteria for growing areas as follows: "The median bacteriological content of samples of water . . . shall not show the presence of organisms of the coliform group [total coliform bacterial density] in excess of 70/100 ml of water."

At the 5th and 6th National Shellfish Sanitation Workshops, participants were in disagreement with the 70/100 ml criterion. Studies[8] [9] had shown that the fecal coliform bacterial group was a more sensitive index of fecal pollution than the total coliform group. This conclusion was based on the fact that, in most instances, the presence of fecal coliform organisms could be correlated with the presence of fecal wastes from humans or other warm blooded animals; conversely, investigators found that large numbers of non-fecal coliforms could be recovered from waters and soils that were remote from any known sources of warm blooded animal pollutants.

The Workshop participants reviewed these study findings and discussed coliform standard for shellfish meats and a coliform standard for the waters in which the shellfish are grown. An attempt

was made by some participants to convince the Workshop that the fecal coliform organisms were a better indicator of water pollution than total coliform bacteria. Indicator levels of fecal coliform bacteria were suggested for approved shellfish growing areas by those who favored changing the bacteriological criterion. After considerable consternation among the 1964 and 1968 Workshop representatives, they recommended continued use of coliform organism criteria and further study of the fecal coliform group in shellfish harvesting waters.

The substitution of the fecal coliform indicator group for the total coliform group in judging shellfish harvest area sanitation came under discussion again at the 7th National Shellfish Sanitation Program (NSSP) in 1971.10/ During the Microbiology Task Force deliberations at the Workshop, median fecal coliform values of 7.8 and 43 were recommended as growing area standards by representatives of Maryland and Virginia, respectively. A spokesman from Lousiana remarked that in his State a 23 fecal coliform MPN median standard would close a high percent of "approved" shellfish-growing areas which could not be shown to be affected by known sources of pollution. Delegates from Rhode Island proposed that the words "not more than 10 percent of the samples ordinarily exceed an MPN of 230 per 100 ml" be omitted from the coliform standard. These actions indicate the diversity of opinions expressed by scientists who were actively trying to reevaluate bacteriological criteria for approved shellfish-growing areas. After considerable discussion, all but two State representatives in the 1971 NSSP Task Force voted to support the following FDA recommendation:

"The Food and Drug Administration (FDA) recommends to the 7th National Workshop that the bacteriological criteria for shellfish harvest areas be maintained as stated in the 1965 Revision of the NSSP Manual of Operations [not to exceed a median total coliform value of 70/100 ml[11/]]. It is also recommended that the NSSP continue to use the fecal coliform test as defined in Recommended Procedures for the Examination of Seawater and Shellfish, APHA 1970, as an adjunct to the coliform test to provide additional information relative to water quality of shellfish harvest areas."

The FDA based this recommendation on five conclusions.

1. The present bacteriological criteria for approved growing areas has been tested for many years and has demonstrated its effectiveness in providing safe shellfish.
2. The 70/100 ml value of the coliform standard is well above the lower limits of sensitivity of the multiple tube fermentation procedure. Acceptable levels for a fecal coliform standard would be in the range of the lower limits of sensitivity of the multiple tube fermentation test resulting in more indeterminate values of water quality data.
3. The coliform indicator group is a broad spectrum indicator of pollution compared to the species of organisms recovered by the fecal coliform test.
4. The relationship of numbers of fecal coliform organisms to direct fecal sources has not been established.

5. At the present time, there is no universal agreement among public health experts as to the advisability of accepting a fecal coliform growing area criterion for shellfish growing areas.

THE MOBILE BAY SHELLFISH HARVEST PROBLEM

The Proceedings of the Mobile Bay Enforcement Conference of 1970 contain a discussion of the problems affecting shellfish harvesting in Mobile Bay.[12/] It was reported that during the spring, flood runoff (532,000 cfs) from the Tombigbee-Mobile River system transported large numbers of coliform bacteria into Mobile Bay. The sanitary significance of these coliform densities was unknown; yet, shellfishing areas in Mobile Bay were closed by the State of Alabama during portions of 8 of the past 16 years because the bacterial densities over the shellfish beds exceeded the 70/100 ml total coliform criterion for harvesting. The region has suffered substantial economic losses because of these closures.

The problem was reiterated at a Mobile Bay Technical Committee meeting at Dauphin Island, Alabama on March 24, 1972 (Appendix C). Committee spokesmen for the State of Alabama contended that the application of the total coliform criterion unduly penalized the Gulf Coast of the Southeastern Unived States. Coastal areas have a potential for the occurrence of certain bacteria of the coliform group in sources of no sanitary sgnificance without concomitant growth of enteric pathogens. The Committee was reminded by representatives from

the State of Alabama that during the past decade, portions of Mobile Bay oyster reefs were closed on nine occasions for average periods of 150 days. As of March 24, 1972, a closure (effective February 4, 1972) resulted in a dockside economic loss of nearly $100,000. This was equivalent to a total economic loss to the Mobile area of almost $400,000.

A SUBSTITUTE BACTERIOLOGICAL CRITERION

During the Technical Committee deliberations, opinions were unanimous that a substitute bacteriological criterion for the total coliform test was needed for evaluating shellfish harvest areas in Mobile Bay. The Committee agreed that representatives of EPA and FDA who are responsible for criteria development should consider the following characteristics in the design of a substitute bacteriological criterion.

The substitute bacterial indicator should: (1) be more specific for fecal pollution than the currently used indicator group; (2) stand on its own merit as an indicator of actual sources of fecal pollution without a dependency upon a specific relationship with the coliform group; (3) be more indicative of potential pathogen contamination; (4) consistently indicate the sanitary quality of shellfish-growing waters in diverse geographical locations; and (5) have a survival time in the estuary consistent with that of bacterial pathogens regardless of nutrient value of the water.

After considering several avenues of approach, the Technical Committee agreed on the following recommendation.

TECHNICAL COMMITTEE RECOMMENDATION

Revised October 5, 1972

The Technical Committee recognizes that a median level of 70 total coliforms per 100 ml (with not more than 10 percent of the samples to exceed 230 MPN/100 ml) has been established as the acceptable bacterial level to permit the classification of a shellfish growing area as approved. Administrative procedures permit waiver of this limit if it can be shown by detailed sanitary survey that observed total coliform levels are not of sanitary significance.

Accordingly, the Technical Committee recommends that appropriate Federal and State agencies be allowed the option of utilizing other bacteriological examinations that provide a more meaningful interpretation of the sanitary quality of shellfish growing waters. The Committee further recommends that steps be taken to allow the alternate use of the total coliform group or the fecal coliform group (44.5 $\pm$ 0.1°C) as bacterial indicators. The Technical Committee also recommends that an alternate fecal coliform indicator group, not to exceed a median MPN of 23/100 ml (with not more than 10 percent of the samples to exceed 50 MPN/100 ml) be acceptable for classifying shellfish areas as approved.

Furthermore, the Technical Committee recommends that the Environmental Protection Agency, the Food and Drug Administration, and State shellfish sanitation control agencies combine their efforts to support planning studies and the research needed to develop a rapid, reliable method for measuring significant numbers of *Escherichia coli* and to compare these values with other pollution indicator bacterial densities in estuarine waters used for shellfish harvesting.

The Committee recommends that planning studies be initiated within FY 1973.

LITERATURE CITED

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11. USPHS, 1965. *National shellfish sanitation program, manual of operations, Part III Public Health Service appraisal of state shellfish sanitation programs.* U. S. Department of Health, Education and Welfare, Washington, D.C.

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APPENDIX A

REQUEST FOR THE ESTABLISHMENT

OF THE

MOBILE BAY TECHNICAL COMMITTEE

ENVIRONMENTAL PROTECTION AGENCY

Date: November 30, 1971

Reply to Attn of: Acting Director, Enforcement Division
Region IV

Subject: Mobile Enforcement Conference Recommendation, In Re Shellfish Technical Committee

To: Director, DFI-Denver Center

In accordance with our telephone conversation sometime back and the follow-up conversation we had during our visit to Memphis in September, I was directed by Mr. Stein to advise you that it was your responsibility to see that recommendation 6 of the enclosed conclusions and recommendations for the Mobile Bay Enforcement Conference is followed.

As I indicated to you, when the project summary was approved on March 2 of 1971 after a lengthy delay, the Regional Office had moved to establish a technical committee in compliance with the above referenced recommendation. However, in contacting personnel in Mr. Stein's office, I was advised that this committee was to be established at the national level and that it would be coordinated in Washington. Vic Lambou started working on this but never got further than preliminary discussions with HEW. After the standards section was removed from the Enforcement Office, Lambou ceased working on the project.

Also as indicated to you, Jim Silva at my request prepared a charter and developed a list of prospective committee members (copy enclosed).

You will note in the recommendation that the committee is to report to the conferees within a year. Because of the delay in getting approval of the conference summary, the due date for the committee's report is March 2, 1972.

Your early attention to this matter will be appreciated.

John C. White

John C. White

Enclosures: Conclusions & Recommendations
Charter & Member List

VIRONMENTAL PROTECTION AGE
OFFICE OF ENFORCEMENT
DIVISION OF FIELD INVESTIGATIONS – DENVER CENTER
BUILDING 22 , ROOM 410 , DENVER FEDERAL CENTER
DENVER, COLORADO 80225

TO : Mr. John White, Acting Director,
Enforcement Division, Reg. IV, EPA
Atlanta

DATE: December 6, 1971

FROM : Director, DFI - Denver Center

SUBJECT: Establishment of Shellfish Technical Committee -
Mobile Bay

I have received your memorandum of November 30, 1971, requesting establishment of a committee to recommend acceptable bacteriological criteria for Mobile Bay. This request from your office is made to assure compliance with Recommendation No. 6 of the Mobile Bay Enforcement Conference.

I am sure you recognize the delay in transmitting this request effectively precludes completion of the required report by March 2, 1972. I am asking Mr. Robert Schneider, Chief, Biology Branch, to serve as representative from this office on the prospective committee. We have also requested that Mr. David Clem, Director, Division of Shellfish Sanitation, serve as the DHEW representative. At this time I would like to request the services of Jim Silva of the Atlanta Regional Office, EPA, as the regional representative. It is my intention that these people should meet at the earliest mutual opportunity to designate an industrial and state representative. Recommendations of this committee should be based upon considerations relevant to Mobile Bay only. It is presently anticipated that the committee will submit a progress report to the Conferees by March 1972.

Your early attention to this proposal will be appreciated and we look forward to working with you and your excellent staff in completing this assignment in a timely manner. You will be kept fully informed of committee activities. If you have any further questions, please do not hesitate to contact me.

Thomas P. Gallagher

cc: Adm., Reg. IV, EPA
Dir., Enf. Proceedings Div., EPA, w/cy
memo, Act. Dir., Enf. Div., Reg. IV,
11/30/71, same subject
Chief, Bio. Br., DFI, w/memo & encl. ✓
Dir., Div. of Shellfish San., DHEW

December 6, 1971

Mr. David Clem, Director
Division of Shellfish Sanitation
DHEW, 200 "C" St., S.W.
Washington, D. C. 20204

Dear Mr. Clem:

As you know, Recommendation No. 6 of the Mobile Bay Enforcement Conference directed that a technical committee be established to develop a more realistic criterion than the present method to more effectively evaluate the probability of pathogen presence in Mobile Bay. This responsibility has been assigned to Division of Field Investigations Center - Denver, EPA.

I have designated Mr. Robert Schneider as this Division's representative on this committee and I would like to solicit you as a representative also. I have asked Mr. Schneider to be in touch with you to arrange a meeting at the earliest convenience. A copy of my letter to the Regional Office is also enclosed. I believe that your participation on this committee is absolutely essential to the development of relevant recommendations to the Conferees.

Your early attention to this matter will be greatly appreciated.

Sincerely,

Thomas P. Gallagher
Director

encl. (1)
Cy memo 12/6/71 to Act. Dir.,
Enf. Div., Reg. IV

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
WASHINGTON, D.C. 20204

December 14, 1971

Mr. Thomas P. Gallagher
Director
Environmental Protection Agency
Office of Enforcement
Division of Field Investigations
Building 22, Room 410
Denver, Colorado 80225

Dear Tom:

This replies to your letter of December 6 regarding the establishment of a technical committee to consider recommendation No. 6 of the Mobile Bay Enforcement Conference. I would be pleased to participate on this committee and to attend a meeting of the committee in early 1972. I would also like to request that Mr. Daniel A. Hunt, Assistant Director, be appointed as my alternate to the committee.

I will look forward to hearing from you or Mr. Robert Schneider regarding the time and place of the meeting.

Sincerely yours,

J. David Clem
Director
Division of Shellfish Sanitation (BF-230)
Office of Food Sanitation
Bureau of Foods

ENVIRONMENTAL PROTECTION AGENCY

Date: December 23, 1971

Reply to Attn of: Director, Enforcement Division
Region IV

Subject: Establishment of Shellfish Technical Committee--Mobile Bay

To: Director, DFI - Denver Center

The proposal set forth in your memorandum of December 6 concerning the above subject is satisfactory.

Mr. Traina has authorized Mr. Jim Silva to serve as regional representative on the committee.

John C. White

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENFORCEMENT
DIVISION OF FIELD INVESTIGATIONS – DENVER CENTER
BUILDING 22 . ROOM 410 . DENVER FEDERAL CENTER
DENVER, COLORADO 80225

4 January 1972

J. David Clem, Director
Division of Shellfish Sanitation
Office of Food Sanitation
Bureau of Foods
Washington, D.C.

Dear Dave:

The first meeting of the Technical Committee to consider recomaendation No. 6 of the Mobile Bay Enforcement Conference will be held in:

Environmental Protection Agency
Region IV Office
3rd Floor
1421 Peachtree Street, NE
Atlanta, Georgia

We will meet in Mr. Jim Silva's office at 1:00 p.m. on Tuesday, January 18, 1972. I have prepared a charge that we will discuss at this first meeting.

I will look forward to seeing you in Atlanta.

Sincerely,

Robert F. Schneider, Acting Chief
Biology Branch

RFS:ls

cc: T. P. Gallagher, Director, DFI-DC
F. J. Silva, Atlanta Regional Office

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENFORCEMENT
DIVISION OF FIELD INVESTIGATIONS – DENVER CENTER
BUILDING 22, ROOM 410, DENVER FEDERAL CENTER
DENVER, COLORADO 80225

COPY

TO : Mr. F. J. Silva
Atlanta Regional Office

DATE: 12 January 1972

FROM : Chief, Biology Section

SUBJECT: Postponement of Technical Committee to Study Mobile Bay Enforcement Conference Recommendation No. 6.

Following our telephone conversation of January 10, 1972 I contacted Mr. Clem to advise him of the need to postpone our Technical Committee Meeting until February. He has arranged his calendar accordingly.

As it is set up now, we will meet with you in your Atlanta Office at 1:00 p.m. on Tuesday, February 1, 1972. There will be five of us at this first meeting as follows:

Committee Members

Robert Schneider - DFI-DC
Jim Silva - Atlanta Region - EPA
Dave Clem - FDA Headquarters

Others

Bobby Carroll - SEWL - EPA
Roger Olmsted - Atlanta Region - FDA

Thanks for letting me know of your January calendar conflict. I am looking forward to our February meeting.

R. F. Schneider

RFS/lms

cc: T. P. Gallagher, DFI-DC
J. D. Clem, FDA, Washington, D.C.
B. J. Carroll, SEWL, Athens, GA

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Regional Food & Drug Director (ATL-F1)

DATE: January 17, 1972

ATTN: Chief, Special Programs Branch (ATL-F15)

FROM : Director
Division of Shellfish Sanitation (BF-230)
Office of Food Sanitation/Bureau of Foods

SUBJECT: First Meeting of the Technical Committee on Recommendation 6 of the Mobile Bay Enforcement Conference

1. This is to confirm my plans to visit the Regional Office, February 1, in connection with a meeting of the technical committee organized to consider recommendation No. 6 of the Mobile Bay Enforcement Conference. I suggest that Mr. Roger Olmsted accompany me to the meeting that has been scheduled for 1:00 p.m. in Mr. Jim Silva's office. I plan to visit your office, opening of business, Feb. 1.

J. David Clem

cc: R. Schneider, EPA, Colorado
T. Gallagher, DFI-DC
F. Silva, ATL Regional Office
R. Olmsted, ATL Regional Office

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENFORCEMENT
DIVISION OF FIELD INVESTIGATIONS – DENVER CENTER
BUILDING 22 , ROOM 410 , DENVER FEDERAL CENTER
DENVER, COLORADO 80225

10 February 1972

Mr. James McDermott
1921 Jefferson Davis Highway
Arlington, Virginia 20242

Dear Mr. McDermott:

Through the Environmental Protection Agency - Office of Enforcement, I have been requested to establish a committee to recommend acceptable bacteriological criteria for Mobile Bay shellfish harvesting areas. I would like to solicit the services of Mr. C. B. Kelly of your staff to join this committee as a representative for Water Hygiene.

As a way of giving you some background, I will briefly describe the history behind this request. On January 27 and 28, 1970 a Conference called by the Secretary of the Interior was held in Mobile, Alabama concerning the economic effects of pollution in Mobile Bay upon the Alabama shellfish industry. Recommendation No. 6 of the "Proceedings--Conference in the Matter of Pollution Affecting Shellfish Harvesting in Mobile Bay, Alabama" deals with shellfish growing water criteria. The recommendation reads as follows: "In determining acceptability of areas in Mobile Bay for shellfish harvesting, a more realistic criterion than the present methods [total coliform density of 70 per 100 ml] be employed to more effectively evaluate the probability of pathogen presence. A coordinated effort by the U. S. Department of Health, Education and Welfare and the U. S. Department of the Interior (now E.P.A. responsibility) in cooperation with the states should be undertaken immediately to develop such a criterion with respect to approval of areas for shellfish harvesting. A technical committee shall be established by the Conferees to assist in the development of such a criterion."

Currently, the technical committee membership consists of: Robert F. Schneider - Committee Chairman; F. James Silva - E.P.A. Regional Representative; J. David Clem - DHEW Representative.

At a recent committee organization meeting in Atlanta, Georgia Mr. Kelly's name was suggested as a committee member to represent Water Hygiene. Because of his vast experience in microbiology and knowledge of shellfish problems I believe his participation on the technical committee is essential to successful development of relevant recommendations to the Conferees.

Arrangements are being made at the Water Hygiene Dauphin Island facility in Mobile, Alabama for a technical committee meeting place. The first meeting of the technical committee is scheduled for March 23, 1972. All committee members will be provided with a specific schedule as soon as arrangements are final.

Your early attention to this matter will be greatly appreciated.

Sincerely,

Robert F. Schneider

Robert F. Schneider
Chief, Biology Branch

RFS/ls

bc: Mr. Kelly

MAR 1972
RECEIVED
DIV. OF FIELD INVESTIGATIONS-DVR. CTR.

ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 2 - 1972

Mr. Robert F. Schneider
Chief, Biology Branch
Division of Field Investigations - Denver Center
Office of Enforcement
Environmental Protection Agency
Denver, Colorado

Dear Mr. Schneider:

I shall be pleased to assign Mr. C. B. Kelly to serve on the Technical Committee to develop criteria of quality for shellfish growing areas. He will be prepared to attend a meeting of the committee to be held at Dauphin Island, Alabama on March 24, 1972.

Sincerely yours,

James H. McDermott, P.E.
Director
Water Supply Programs Division

15 February 1972

Mr. C. D. Kelly
Special Assistant for Research
Division of Criterion Standards
Water Supply Program Division
Room 500, Crystal Mall Building 2
Washington, D.C. 20460

Dear Mr. Kelly:

On behalf of the Technical Committee I would like to welcome you as a member. I am looking forward to meeting with you and others of the Technical Committee to discuss our task of developing a more realistic criterion for shellfish growing areas in Mobile, Alabama.

The Technical Committee membership is as follows:

1) J. David Clem, FDA Representative;
2) F. James Silva, EPA Regional Representative;
3) Arthur Beck, Alabama Water Improvement and Health Department Representative;
4) James Coff, Pulp and Paper Industry Representative; and
5) W. B. Anderson, Alabama Conservation and Natural Resources Representative.

A Technical Committee meeting is being planned and will be held at the E.P.A. Water Hygiene Laboratory, Dauphin Island, Mobile, Alabama on March 24, 1972. You will be provided with a specific standard when arrangements are final.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

14 February 1972

Mr. Arthur M. Beck, Technical Secretary
Water Improvement Commission
State Office Building
Montgomery, Alabama 36104

Dear Mr. Beck:

On behalf of the Technical Committee I would like to welcome you as a member. I am looking forward to meeting with you and others of the Technical Committee to discuss our task of developing a more realistic criteria for shellfish growing areas in Mobile Bay.

A Technical Committee meeting is being planned and will be held in the F. D. A. Water Hygiene Laboratory, Dauphin Island, Mobile, Alabama on March 24, 1972. You will be provided with a specific schedule as soon as arrangements are final.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/le

14 February 1972

Mr. James Coil
Manager of Administrative Services
Scott Paper Company
P. O. Box 2447
Mobile, Alabama 36601

Dear Mr. Coil:

Pursuant to our telephone conversation of February 11, 1972 may I take this opportunity to welcome you to the Mobile Bay Technical Committee. I am looking forward to meeting with you and others of the Committee to discuss our challenging task of recommending acceptable bacteriological criteria for shellfish areas in Mobile Bay.

A Technical Committee Meeting is being planned and will be held at the E. P. A. Water Hygiene Laboratory, Dauphin Island, Mobile, Alabama on March 24, 1972. You will be provided with specifics as soon as arrangements are final.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

APPENDIX B

PROGRESS REPORT

PROGRESS REPORT

MOBILE BAY TECHNICAL COMMITTEE

R. F. Schneider

On January 27-28, 1970 in the City of Mobile, Alabama, a Conference in the Matter of Pollution Affecting Shellfish in Mobile Bay, Alabama was called by the Secretary of the Interior. Recommendations contained in the Conference Proceedings included one that emphasized reevaluation of the bacteriological criterion for shellfish harvesting areas.

Recommendation No. 6 reads: "In determining acceptability of areas in Mobile Bay for shellfish harvesting, a more realistic criterion than the present method be employed to more effectively evaluate the probability of pathogen presence. A coordinated effort by the U. S. Department of Health, Education and Welfare and the U. S. Department of the Interior, in cooperation with the States, should be undertaken immediately to develop such a criterion with respect to approval of areas for shellfish harvesting. A technical committee shall be established by the Conferees to assist in the development of such a criterion. This committee should report to the Conferees no later than one year from the date of the first session of conference."

In compliance with Recommendation No. 6 of the Mobile Bay Enforcement Conference the following persons agreed to serve on the Technical Committee: Mr. Robert Schneider, NFIC-Denver

Mr. J. David Clem, DHEW, Washington, D.C.

Mr. F. James Silva, EPA, Atlanta, Georgia

On February 1, 1972, the members listed above met in Atlanta, Georgia with Mr. Bobby Carroll, Chief Bacteriologist, EPA, Southeastern U. S. Region and Mr. Roger Olmstead, FDA, Southeastern U. S. Region. This meeting had a multi-purpose: (1) recommend prospective representatives from the pulp and paper industry, fishery industry and the State of Alabama; (2) review the highlights of the 1970 Mobile Bay Enforcement Conference; (3) discuss the rationale behind the present bacteriological criterion for shellfish growing areas and (4) jointly review the charge to the technical committee.

Suggested additions to the Technical Committee membership included: Mr. C. B. Kelly of EPA to represent the Water Hygiene Program; Mr. James Coil of Scott Paper Company to represent the pulp and paper industry; Mr. Arthur Beck, the Chief Sanitary Engineer of Alabama to represent the Alabama Water Improvement Commission and the Alabama Health Department; and Mr. W. F. Anderson, Chief of the Alabama Marine Resources to represent the Alabama Department of Conservation and Natural Resources. These individuals have agreed to serve on the Technical Committee in the capacity noted above.

Highlights of the 1970 Enforcement Conference Proceeding and the rationale behind the total coliform standard for shellfish growing areas were reviewed by attendees at the meeting in Atlanta, Georgia on February 1, 1972. Discussions centered around the fact that during the late winter or spring, flood runoff from the Tombigbee-Mobile River system transports large coliform concentrations into Mobile Bay. The

sanitary significance of these total coliform densities is unknown; yet, shellfishing areas in Mobile Bay have been closed by the State of Alabama because the bacterial densities over the shellfish beds exceeded the 70/100 ml total coliform standard for harvesting. The region has suffered substantial economic losses because of these closures.

The most recent closure is in effect, currently. On January 28, 1972 the State of Alabama closed all areas of Mobile Bay east of Dauphin Island Bridge because the total coliform density exceeded the 70/100 ml criterion. This closure means that some of the most productive shellfish areas of the Bay (Bon Secour Bay) are closed to harvesting for at least a portion of the 1972 season. Economic loss to the region because of past closures (excluding the 1972 closure) is estimated to exceed 200 thousand dollars.

After reviewing and discussing Recommendation No. 6 and other shellfish related problems in Mobile Bay the following charge to the Technical Committee was developed:

"The Technical Committee is requested to assist in the development of a more realistic bacteriological criterion than the present method [total coliform density of 70/100 ml] for determining the probability of pathogen presence in Mobile Bay shellfish harvesting waters. To accomplish this goal the Committee may form such sub-committees as needed to review various avenues of approach, provide technical information or conduct studies. The Committee will select an approach and will set into motion a scheduled program to fulfill its charge. Furthermore, the Committee may exercise such latitude as required in fulfilling

its charge and shall meet at such times and places as necessary."

In response to this charge the Technical Committee has agreed to meet in Mobile, Alabama on March 24, 1972. Various bacteriological criteria for determining the acceptability of Mobile Bay waters for shellfish harvesting will be discussed. Consideration will be given to the sanitary significance of fecal coliform, fecal streptococci, FC/FS ratio, or other parameters that could be used for evaluating shellfish harvesting waters. The Committee will appoint a subcommittee to review technical reports concerning the "State of the Art" and sanitary significance of the various bacteriological criteria. The subcommittee should be prepared to submit a written report of its findings to the Technical Committee by August 1, 1972. After reviewing the subcommittee findings, the Technical Committee will meet at their earliest opportunity (no later than September 15, 1972) to formulate a recommendation. The Committee chairman will prepare and submit the final recommendation to the Conferees by October 1, 1972.

APPENDIX C

MOBILE BAY TECHNICAL COMMITTEE MEETING

DAUPHIN ISLAND, ALABAMA

MARCH 24, 1972

1 March 1972

Mr. James Coll
Manager of Administrative Services
Scott Paper Company
P.O. Box 2447
Mobile, Alabama 36601

Dear Mr. Coll:

The technical committee to consider recommendation no. 6 of the Mobile Bay Enforcement Conference will meet in Mobile, Alabama on March 24, 1972. The meeting is scheduled for 9:00 a.m. in the library (conference room) of the Water Hygiene laboratory located on Dauphin Island. If you need specific directions, please contact: Richard J. Hammerstrom, Director
Gulf Coast Water Supply Laboratory
EPA
P.O. Box 158
Dauphin Island, Alabama 36528
Phone: 205/861-2962 (not an FTS number)

At the meeting we plan to discuss several bacteriological criteria for determining the acceptability of Mobile Bay waters for shellfish harvesting. It would be helpful to the committee if you would present your views on the use of fecal coliform tests (temperature elevated bacteria) versus E coli (IMVIC) tests or other alternate methods to determine sanitary conditions in shellfish harvesting areas.

I am looking forward to working with you on this technical committee.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

1 March 1972

Mr. J. David Clem
Division of Shellfish Sanitation
Bureau of Foods
Washington, D.C.

Dear Dave:

Plans have been finalized for our technical committee meeting. We will meet at the Water Hygiene Laboratory on Dauphin Island at 9:00 a.m. on March 24, 1972.

Because four of the committee members were absent from the Atlanta meeting, there will be a need for repeating some of our previous discussions. Would you discuss the rationale behind the present bacteriological criterion for shellfish growing areas as you did for us in Atlanta?

I will be looking forward to working with you again on March 24.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

1 March 1972

Mr. F. J. Silva
EPA, Region IV
Suite 300
1421 Peachtree Street, NE
Atlanta, Georgia 30309

Dear Jim:

Our technical meeting is set for 9:00 a.m. on March 24 at the Water Hygiene Laboratory, Dauphin Island, Alabama. Thanks for all your special efforts as I mentioned in our recent telephone conversation. I do have one more favor to ask of you. Because four of the committee members were absent from the Atlanta meeting, there will be a need for repeating some of our previous discussions. Would you review the highlights of the 1970 Mobile Bay Enforcement Conference? This would give the members of our full committee a common background.

I'll be looking forward to our Dauphin Island meeting with the opportunity to work with you on this long overdue shellfish standard revision.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

1 March 1972

Mr. Arthur H. Beck, Technical Secretary
Water Improvement Commission
State Office Building
Montgomery, Alabama 36104

Dear Mr. Beck:

The technical committee to consider recommendation no. 6 of the Mobile Bay Enforcement Conference will meet in Mobile, Alabama on March 24, 1972. The meeting is scheduled for 9:00 a.m. in the library (conference room) of the Water Hygiene laboratory located on Dauphin Island. If you need specific directions please contact: Richard J. Hammerstrom, Director
Gulf Coast Water Supply Laboratory
EPA
P.O. Box 158
Dauphin Island, Alabama 36528
Phone: 205/861-2962 (not an FTS number)

Some of the committee members are not familiar with the recent closure of Mobile Bay. At our March 24 meeting would you brief the committee on the Bay closure and discuss the State of Alabama views on the sanitary significance of the present bacteriological criterion?

I am looking forward to working with you on this special technical committee.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

1 March 1972

Mr. C. B. Kelly
Special Assistant for Research
Division of Criterion Standards
Water Supply Program Division
Room 500, Crystal Mall Building 2
Washington D.C. 20460

Dear Mr. Kelly:

The technical committee to consider recommendation no. 6 of the Mobile Bay Enforcement Conference will meet in Mobile, Alabama on March 24, 1972. The meeting is scheduled for 9:00 a.m. in the library (conference room) of the Water Pollution Laboratory located on Dauphin Island. If you need specific directions please contact: Richard J. Hamerstrom, Director
Gulf Coast Water Supply Laboratory
EPA
P.O. Box 158
Dauphin Island, Alabama 36528
Phone: 205/861-2262 (not an FTS number)

At the meeting we plan to discuss several bacteriological criteria for determining the acceptability of Mobile Bay waters for shellfish harvesting. It would be helpful to the committee if you would present your views on the sanitary significance of various bacteriological indicators such as fecal coliform.

I am looking forward to meeting you and having the opportunity to work together on developing a recommendation for the Conferees.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

2 March 1972

Mr. W. B. Anderson
Department of Conservation and Natural
Resources
Marine Resources Division
P.O. Box 189
Dauphin Island, Alabama 36528

Dear Mr. Anderson:

The technical committee to consider recommendation no. 6 of the Mobile Bay Enforcement Conference will meet in Mobile, Alabama on March 24, 1972. The meeting is scheduled for 9:00 a.m. in the library (conference room) of the Water Hygiene Laboratory located on Dauphin Island. If you need specific directions please contact:

Richard J. Hamerstrom, Director
Gulf Coast Water Supply Laboratory
EPA
P.O. Box 150
Dauphin Island, Alabama 36528
Phone: 205/861-2262 (not an FTS number)

Recently, I learned that Mobile Bay is closed to shellfish harvesting again this season. It would be helpful to the committee if you would present some information concerning the economic loss or other problems to the Mobile area because of this closure.

I'm looking forward to having the opportunity of working with you on this technical committee.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

State of Alabama
Department of Public Health
State Office Building
Montgomery, Alabama 36104

IRA L. MYERS, M. D.
STATE HEALTH OFFICER

March 6, 1972

Mr. Robert F. Schneider
Chief, Biology Branch
Environmental Protection Agency
Water Quality Office
Bldg. 22, Room 410
Denver Federal Center
Denver, Colorado 80225

Dear Mr. Schneider:

Thank you for your letter of March 1, 1972 advising me of the meeting date and place of the newly formed Technical Committee to implement the recommendations of No. 6 of the Mobile Bay Enforcement Conference.

I will be happy to make any contribution that I can to the Committee members at the March 24th. meeting.

Yours very truly,

Arthur N. Beck
Chief Engineer & Director
Bureau of Environmental Health

ANB/iw

Status of Bacteriological Criteria for Shellfish Areas

The fecal coliform indicator group has been under consideration by the National Shellfish Sanitation Program (NSSP) as a substitute for the coliform group in the bacteriological criteria for shellfish-growing areas for a number of years. A fecal coliform standard of 7.8 was proposed at the Fifth National Workshop in 1964 which represented a coliform/fecal coliform ratio of approximately 10/1 when compared to the present coliform standard. There appeared to be an assumption by the Fifth Workshop that any new criteria or standard must have a consistent correlation with the existing coliform standard. The proposed standard was rejected on the basis of insufficient data and additional studies were recommended.

The Microbiology Task Force of the Sixth National Workshop again concluded that there was insufficient evidence for recommending a fecal coliform standard and requested that further work be done. In response to this request, shellfish sanitation personnel have analyzed growing area data from Louisiana, Mississippi, Alabama, Florida, Georgia, Virginia, New York, Rhode Island, Massachusetts, and Maine. These data

Prepared by the Division of Shellfish Sanitation, Bureau of Foods, Food and Drug Administration, Washington, D. C. 20204 for presentation at Mobile Bay Technical Committee Meeting, Dauphin Island, Alabama, March 24, 1972.

indicate that there is no consistent coliform/fecal coliform relationship in estuarine waters. The coliform/fecal coliform ratio was found to vary from 1/1 to 10/1 depending upon such factors as geographic location, meteorological conditions, season, pollution source, distance of sampling station from pollution source (dilution effect) and salinity, nutrients, or bacterial inhibitors of receiving waters. We have not been able to correlate these factors with the actual bacteriological data nor determine how these factors relate to the differences in coliform/fecal ratios. The Division of Shellfish Sanitation concluded that any new indicator criteria should be evaluated according to the sanitary significance of the specific indicator and not according to the relationship between the indicator group being evaluated and the present criteria. In review of the fecal coliform water quality data obtained from State shellfish control programs, very few of these data had been correlated with known pollution sources. It has been very difficult to make concise public health appraisal of these data for lack of in depth sanitary survey information.

During the Microbiology Task Force deliberations at the Seventh National Workshop in October 1971, median fecal coliform values of 7.8 and 43 were recommended as growing area standards by representatives of Maryland and Virginia, respectively.

A spokesman from Louisiana stated that a 23 fecal coliform MPN median would close a high percent of "approved" shellfish-growing areas in his State which could not be shown to be affected by known sources of pollution. Delegates from Rhode Island proposed that for the present coliform standard that "not more than 10 percent of the samples ordinarily exceed an MPN of 230 per 100 ml." be omitted from the present coliform standard. These actions may give you some indication as to the diversity of opinions expressed in this task force. Certainly, one must conclude that there is a National need to reconsider and evaluate bacteriological criteria for approved shellfish-growing areas. After considerable discussion, all but two State representatives present in the task force voted to support FDA's proposal to retain the present growing area standards based upon the coliform indicator group.

On February 17, 1972, a representative of the Division of Shellfish Sanitation participated in an Ad Hoc Committee meeting on bacteriology, which included representatives of the Environmental Protection Agency (EPA), Oregon Dept. of Environmental Quality, the academic community, the medical profession, the National Council on Stream and Air Improvement,

and the pulp and paper mill industry. The significance of Klebsiella pneumonia was discussed. This organism, a member of the coliform and in part the fecal coliform groups was reported found in high numbers in paper mill wastes. It may also be found in other waste effluents high in reducing sugars and other carbohydrates such as the sugar industry, food-processing plants, and some domestic effluents. IMViC reactions will not separate Klebsiella pneumoniae from Enterobacter aerogenes.

In attempting to interpret existing data on Mobile Bay waters, there is no way to determine the percentage of the reported coliform or fecal coliform values that are actually K. pneumoniae. Such information would be valuable to the agency responsible for the classification of shellfish-growing areas in the interpretation of sanitary survey data considering that EPA has reported that 51% of the total coliform waste source contribution to Mobile Bay is from paper mill wastes.

During the Ad Hoc Committee meeting, a number of characteristics of this organism were discussed which are significant to agencies using coliform or fecal coliform criteria in the classification of environmental water quality. These are listed as follows:

a. K. pneumoniae is a member of the coliform group.

b. A large percentage (40-60%) of the K. pneumoniae isolated from environmental samples have been reported to ferment lactose at 44.5°C., and, therefore, would be reported in the indicator system as a fecal coliform type.

c. K. pneumoniae has the same IMViC reactions as Enterobacter aerogenes.

d. K. pneumoniae is heavily encapsulated and has a longer survival potential in environmental waters than enteric pathogens or E. coli.

e. K. pneumoniae can "colonize" environmental waters rich in carbohydrates and reducing sugars (such as paper mill wastes in Mobile Bay).

f. K. pneumoniae is a normal inhabitant of the intestinal tract of warmblooded animals, but is generally found in low numbers compared to E. coli populations.

g. K. pneumoniae is highly resistant to antibiotics and is regarded as a "secondary" pathogen by the medical profession. It usually appears as a pathogen following treatment of other enteric bacterial infections after normal populations of bacterial flora have been modified by antibiotic treatment.

h. The presence of E. coli in the intestinal tract of healthy individuals may restrict the growth of K. pneumoniae and help prevent "colonization" of the individual.

i. The presence of effluents enriched with carbohydrates such as paper mill wastes in shellfish harvest areas can have an adverse affect on the classification of such areas, especially if aftergrowth or "colonization" occurs.

j. Aftergrowth of K. pneumoniae can result in high counts of both coliform and fecal coliform groups which may be totally unrelated to actual pollution sources, representing real health hazards to consumers of shellfish.

In view of the confusion surrounding an "acceptable" value of fecal coliform organisms to be used as a substitute for the present coliform standard, FDA proposed to the Seventh National Workshop that NSSP take a closer look at the more specific 45.5°C test for E. coli. This was first proposed to the American Public Health Association Committee on shellfish methodology by its chairman, Dr. C. A. Perry, in 1936. Goldreich states that the fecal coliform test at 44.5 (40.5°)C represents a, ". . . compromise between acceptable sensitivity and specificity for the fecal types."

For regulatory purposes, the FDA has been successful in the courts in using E. coli as an indicator of fecal pollution. Perhaps, the time has come in the history of the NSSP, that we must re-evaluate our concepts and be willing to lose sensitivity for an increase in specificity in the promulgation of both bacteriological criteria and standards for "approved" shellfish harvest areas. Currently, neither the FDA nor the NSSP has been convinced of the wisdom of substituting the narrow spectrum fecal coliform test for the broad spectrum coliform group.

If we make errors in standard setting, let it be on the side of safety. On the other hand, FDA and cooperating State agencies responsible for growing area control have no desire to prevent utilization of a natural resource by closing harvest areas where indicator levels exceed present standards, unless these indicator levels, according to the best professional knowledge and experience, constitute a real health hazard.

In conclusion, we realize the need for a more specific indicator and more realistic standards for use as an adjunct to the sanitary survey in defining "approved" growing areas. Any substitute for the present criteria should: (1) be more specific for fecal pollution than the current indicator group,

(2) stand on its own merit as an indicator of actual sources of fecal pollution without a dependency upon a specific relationship with the coliform group, (3) be more representative of the potential pathogen load, (4) consistently indicate the sanitary quality of shellfish-growing waters in diverse geographical locations, (5) and have a survival time ratio in the estuary consistent with that of other bacterial pathogens regardless of nutrient value of the water.

As previously stated, the Seventh National Workshop, the NSSP supported the FDA position by a large majority that the fecal coliform has not adequately fulfilled the qualifications necessary for the promulgation of a fecal coliform standard for "approved" shellfish harvest areas. The need for a re-evaluation and renewed effort to develop new criteria is recognized.

We recommend that E. coli be considered as a promising bacteriological indicator for classifying shellfish harvest areas and that necessary research be initiated to develop and/or evaluate rapid tests for the recovery of this organism. The long-term goal would be new bacteriological criteria and standards for the classification of growing areas. It is

our considered opinion that this effort should receive immediate attention and research investment by Federal and State agencies concerned with shellfish sanitation control.

J. D. Clem

D. A. Hunt

March 22, 1972

March 24, 1972

COPY

Economic Loss of Oyster Reef Closure

1971 - 1972

This season Alabama's oyster reefs were closed on January 30 and February 4 (Productive reefs) and have remained closed for the past 50 days.

The average dockside value of the oyster catch is $1,671 or a total loss of $83,550. However, production this yearwas above average and was worth $1,916 per day in December 1971. At this figure the total dockside loss has been $95,800 during the current season.

The dockside value represents only loss to the fisherman. It has been shown that the total economic value of oyster production in Alabama is four times the dockside value. Thus, the total economic loss to the fisheries community as a result of the current pollution closure is $382,400.

Prepared by the Alabama Department of Conservation and Natural Resources for presentation at Mobile Bay Technical Committee Meeting, Dauphin Island, Alabama, March 24, 1972.

Donald W. Lear, 1962, Growth of bacteria in estuarine waters. Chesapeake Science 3 (3): 160-165.

--Organisms used were Escherichia coli, E. freundii, and Aerobacter aerogenes.

--Nutrients were added to filtered distilled water and to filtered estuarine water (14.1 ppt).

--Growth was better in the saline waters than in the distilled waters.

--Cites papers by Kelly and Arcise (1954) which show increase in coliform MPN in oysters shipped--indicating growth in saline conditions utilizing food sources within the oyster.

--Implication is that if nutrient sources are available in saline waters total coliform count will increase.

Gallagher, T.P. and D.F. Spino, 1968, The significance of numbers of coliform bacteria as an indicator of enteric pathogens. Water Research, Vol. 2, 169-175.

The traditional concept of using levels of total coliform densities to describe bacteriological acceptability has been questioned by many because coliforms may result from causes other than fecal pollution. Because fecal coliform densities result from pollution by man and other warm-blooded animals, they are more directly indicative of the probable presence of the enteric associated pathogens. However, summarized data from several stream surveys over the past few years show little apparent correlation between quantities of total or fecal coliforms and the probable isolation of salmonellae. Salmonellae were isolated at total coliform densities of less than 1000/100 ml and fecal coliform densities of less than 150/100 ml. The total coliform density of 1000/100 ml has traditionally been acceptable for recreational waters. It is probable that salmonellae will be isolated at high fecal coliform densities but observed data has indicated that isolation is also probable at low coliform densities if there is an

inadequately disinfected waste source which may affect the area of concern. In one instance cited, the area was 73 miles and 4 days travel time downstream from sources of waste. This may be because salmonellae are persistent under conditions which may be adverse to survival of fecal coliform. Large percentage reductions of coliform without chlorination will not of itself guarantee bacteriological acceptability. Data show that salmonellae have been isolated after a 99 percent reduction in fecal coliform below unchlorinated waste sources.

Although salmonellae at present are the only pathogens which may be isolated from water by means of a routine test with any degree of reliability, it is probable that methods for other pathogens will be perfected in the near future. Until that time, the coliform will still be valuable as indicators of pollution. However, low values of total and fecal coliform are not, of themselves, sufficient to indicate bacteriological safety from pathogenic organisms. A control program to more effectively evaluate bacteriological safety in recreational waters should include: (1) a sanitary survey to delineate possible sources of pollution; (2) effective disinfection of all waste sources which may affect the area; (3) use of the salmonella test in addition to total and fecal coliform as a monitoring device.

Rubin, A. J., et al, 1969, Coagulation of Escherichia coli by neutral salts. Water Research. Vol. 3, 843-852.

Shows E. coli to coagulate at about 23 ppt NaCl (.415N) at a pH of 6. Which is beyond the desirable salinity range for oyster production and indicates only dilution reduces the number of coliforms. This dilution is made by decrease in freshwater flow in relation to saline waters within the bay. pH's in the range of the bay water cause no increase in aggregation or coagulation.

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENFORCEMENT
DIVISION OF FIELD INVESTIGATIONS – DENVER CENTER
BUILDING 22, ROOM 410, DENVER FEDERAL CENTER
DENVER, COLORADO 80225

TO : Files

DATE: 29 March 1972

FROM : Robert F. Schneider
Chief, Biology Branch

SUBJECT: Mobile Bay Technical Committee Meeting, Dauphin Island, Alabama March 24, 1972.

Attendees of the March 24, 1972 Technical Committee Meeting to consider Recommendation No. 6 of the Mobile Bay Enforcement Conference were:

Committee members:	Representing
W. F. Anderson	Alabama Department of Conservation and Natural Resources
James Coil, Jr.	Scott Paper Company, Mobile, Alabama
Arthur Beck	Alabama Water Improvement Commission
J. David Clem	FDA, Washington office
C. B. Kelly	EPA, Washington office
Robert Schneider	Chairman
F. J. Silva (absent because of illness)	EPA, Atlanta office

Others:	
Albert Story	FDA, Dauphin Island, Alabama
Eddie May	Alabama Marine Resources, Dauphin Island, Alabama
Wayne Swigle	Alabama Marine Resources, Dauphin Island, Alabama

Committee member spokesmen for the State of Alabama discussed the present status of shellfish harvesting areas in Mobile Bay. They informed the Committee that the Bay oyster production in December, 1971 was nearly $300.00 per day higher than it had been for the past several years. On February 4, 1972 all productive reefs in Mobile Bay were closed and have remained closed for the past 50 days. Closure has resulted in an economic loss (total dockside) of $95,800. The dockside value represents only loss to the fisherman. Alabama State officials explained that the total economic value of oyster production in Alabama is four times the dockside value; thus, the total economic loss to the fisheries community as a result of the current pollution closure is $382,400.

Discussions during the remainder of the meeting dealt with the various bacteriological criteria that might be substituted for the total coliform criterion presently used in evaluating shellfish harvesting waters. The fecal coliform indicator group was the principal substitute criterion considered. The FDA spokesman told the Committee that neither the FDA nor the NSSP is convinced of the wisdom of substituting the narrow spectrum fecal coliform test for the broad spectrum coliform group. Contrasting views were expressed by other attendees. They pointed out to the Committee that fecal coliform densities result from pollution by man or other warm blooded animals; therefore, the fecal coliform test was more directly indicative of the probable presence of enteric associated pathogens than the "broad spectrum" coliform group.

Although the Committee did not reach an agreement (during this session) on a more specific indicator or more realistic standards for Mobile Bay, we did establish the qualifications any substitute criterion must have. Based upon a prepared statement by the FDA representative, the Committee agreed that the substitute for the present criterion should: (1) be more specific for fecal pollution than the current indicator group, (2) stand on its own merit as an indicator of actual sources of fecal pollution without a dependency upon a specific relationship with the coliform group, (3) be more representative of the potential pathogen load, (4) consistently indicate the sanitary quality of shellfish-growing waters in diverse geographical locations, and (5) have a survival time ratio in the estuary consistent with that of other bacterial pathogens regardless of nutrient value of the water.

Within the Committee, a working committee was established. Committeemen Clem and Kelly agreed to jointly prepare a statement suggesting an approach to assist regulatory agencies in the development of a meaningful bacteriological criterion for Mobile Bay shellfish harvesting waters. The statement will be submitted to the Committee Chairman by April 14, 1972 and subsequently distributed by the Chairman to Committee members for their review and comment.

On May 5, 1972 the Technical Committee will meet at Dauphin Island, Alabama to discuss the statement prepared by the working committee and to deliberate a relevant recommendation to the Conferees.

Robert F. Schneider

Robert F. Schneider

RFS/ls

APPENDIX D

CORRESPONDENCE CONCERNING A PROPOSED RECOMMENDATION

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENFORCEMENT
DIVISION OF FIELD INVESTIGATIONS – DENVER CENTER
BUILDING 22, ROOM 410 , DENVER FEDERAL CENTER
DENVER, COLORADO 80225

TO : Mobile Bay Technical Committee Member

DATE: 24 April 1972

FROM : Robert F. Schneider, Chairman

SUBJECT: Technical Committee to Consider Recommendation No. 6 of the Mobile Bay Enforcement Conference.

Following our deliberations in Dauphin Island, I have had the opportunity to talk with some of you by telephone concerning the Mobile Bay shellfish harvesting problem. From these conversations and from the materials presented to the Committee during our March 24, 1972 meeting I have developed the opinions contained herein.

There is a Committee consensus that a substitute criterion for the total coliform test is needed for evaluating shellfish harvest areas in Mobile Bay. Those who are responsible for criteria development (i.e. FDA shellfish Sanitation Branch and EPA Office of Water Programs) should consider the following characteristics in the design of a substitute bacteriological criterion. The "new" bacterial indicator should: (1) be more specific for fecal pollution than the current indicator group, (2) stand on its own merit as an indicator of actual sources of fecal pollution without a dependency upon a specific relationship with the coliform group, (3) be more representative of the potential pathogen load, (4) consistently indicate the sanitary quality of shellfish-growing waters in diverse geographical locations, (5) and have a survival time ratio in the estuary consistent with that of other bacterial pathogens regardless of nutrient value of the water.

The Committee considered two alternative indicators as the most promising for evaluating bacteriological contamination in Mobile Bay shellfish harvest areas, (1) fecal coliform or (2) Escherichia coli.

Committee members and other attendees at the March 24 Dauphin Island meeting pointed out certain benefits and weaknesses of these substitute indicators. The major points were:

Presently, the fecal coliform test requires less time and effort than the E. coli measurement; therefore, results can be obtained sooner, at less expense, and by fewer microbiologists. However, studies by the Gulf Coast Laboratory and others have failed to demonstrate a

consistent total coliform/fecal coliform ratio or arrive at a fecal coliform value that can be directly related to known sources of fecal pollution and provide public health protection to consumers of raw shellfish.

Sanitary surveys and published documentation confirm the fact that the presence of E. coli consistently indicates fecal pollution and the potential contamination by other pathogenic bacteria.

Based upon these considerations and in compliance with Recommendation No. 6 of the Mobile Bay Enforcement Conference, the Technical Committee submits the following statements to the Conferees: "To assist in the development of a more realistic criterion than the present method for evaluating the probability of pathogen presence in Mobile Bay, it is recommended that the criterion be based on the measurement of Escherichia coli in waters overlying shellfish harvest areas. Furthermore, the Technical Committee recommends that the Federal and State shellfish sanitation control agencies combine their efforts to support the research needed to develop a rapid, reliable method for enumerating E. coli in estuary waters used for shellfish harvesting."

At present, I suggest we postpone the tentative meeting scheduled at Dauphin Island on May 5, 1972. I would like to give each of you the opportunity to respond to the recommendation contained herein. If possible, I would like your written comments by May 10, 1972. If you feel further Technical Committee meetings would be fruitful, please advise me and I will set up a mutual meeting place and time.

Bob Schneider

Robert F. Schneider

RFS/ls

cc: Mr. John White, Atlanta, GA
Mr. Murray Stein, D.C.

STATE OF ALABAMA

DEPARTMENT OF CONSERVATION AND NATURAL RESOURCES

P. O. Box 188 - Dauphin Island, Alabama 36528

GEORGE C. WALLACE
GOVERNOR
CLAUDE D. KELLEY
COMMISSIONER
HARRY B. BLEDSOE
ASSISTANT COMMISSIONER

DIVISION OF MARINE RESOURCES
WILLIAM F. ANDERSON, DIRECTOR

April 28, 1972

Mr. Robert F. Schneider, Chairman
Mobile Bay Technical Committee
Environmental Protection Agency
Office of Enforcement
Division of Field Investigations
Building 22, Room 410
Denver Federal Center
Denver, Colorado 80225

Dear Mr. Schneider:

We concur with your opinions 100 percent. Originally we felt that fecal coliform would be a better standard. However, after learning that Klebsiella is detected in the test for fecal coliform and that it also is frequently associated with paper mill effluent, we certainly agree that E. coli would be a much better indicator organism. We feel this way because the discharge of paper mill effluent at the head of Mobile Bay has a population equivalent in excess of 400,000.

It was suggested by some of the FDA research group here on the island that a salinity level of 10 ppt be used as the closure point. However, on examination of some of the data compiled by them (attached) there seems to be little correlation between this level and high coliform levels. Also their supposition is still based on the 70 MPN/10 cc of total coliform in relation to salinity. We, therefore, feel that the criterion you have suggested would be much better.

We are sending under a separate cover some of our publications on Mobile Bay which might be helpful to you for background information.

Sincerely,

W. F. Anderson, Director
Marine Resources Division

WFA:jb

cc: Mr. Claude Kelley/w copy of Mr. Schneiders letter
Mr. Arthur N. Beck

9 May 1972

Mr. W. F. Anderson
Marine Resources
Department of Conservation
and Natural Resources
P.O. Box 189
Dauphin Island, Alabama 36528

Dear Mr. Anderson:

Thank you for your prompt reply to my letter of April 24, 1972. I have not received opinions from all the Committee members yet, but the responses from FDA and Scott Foser are in agreement with the 'E. coli Indicator' recommendation. When I receive the others I will draft a final recommendation and send it to you along with the letters I've received from the Committee for your review and comment.

Again, thank you for your help and for sending me the series of Alabama Marine Bulletins and oyster resource atlas.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
WASHINGTON, D.C. 20204

May 5, 1972

Mr. Robert F. Schneider
Chairman
Mobile Bay Technical Committee
Room 410, Building 22
Denver Federal Center
Denver, Colorado 80225

Dear Bob:

I concur with your letter of April 24 but would like to make the observation that one study conducted by our staff indicates that E. coli can be recovered within 24 hours with results comparable to present methods. Dr. Wallace H. Andrews' paper on this study is attached and has also been published in Applied Microbiology, Vol. 23, No. 3, March 1972 issue. Of course, their work needs further collaboration but it does look promising.

If I can be of any further assistance, please call.

Sincerely,

J. David Clem
Director
Division of Shellfish Sanitation
BF-230
Office of Food Sanitation
Bureau of Foods

Enclosure

SCOTT PAPER COMPANY
SOUTHERN OPERATIONS
P. O. BOX 2447
MOBILE, ALABAMA 36601

JAMES H. COIL, JR.
MANAGER OF
ADMINISTRATIVE SERVICES

May 5, 1972

Mr. Robert F. Schneider
Environmental Protection Agency
Office of Enforcement
Division of Field Investigations-Denver Center
Building 22, Room 410, Denver Federal Center
Denver, Colorado 80225

Dear Mr. Schneider:

Subject: Technical Committee to Consider Recommendation No. 6 of the Mobile Bay Enforcement Conference.

I would suggest consideration of altering your statement to the conferees to read as follows:

"To assist in the development of a more realistic criterion than the present method for evaluating the probability of pathogen presence in Mobile Bay, it is recommended that the criterion be based on the measurement of Escherichia coli in waters overlying shellfish harvest areas. The Technical Committee further recommends that the agencies concerned, i.e., FDA Shellfish Sanitation Branch, EPA Office of Water Programs and the Alabama State Department of Health, initiate as soon as possible surveys of the E. coli counts—in addition to total coli/ counts—in the waters overlying the shellfish areas and on the shellfish proper. Furthermore, the Technical Committee recommends that those aforementioned federal and state shellfish sanitation control agencies combine their efforts to support the research needed to develop a rapid, reliable method for enumerating E. coli in estuary waters used for shellfish harvesting."

Very truly yours,

James H. Coil, Jr.
Paper Industry Representative
Mobile Bay Technical Committee

JHC:por
cc: Mr. John White, Atlanta, GA
Mr. Murray Stein, D. C.

22 410

11 May 1972

Mr. James Coil
Manager of Administrative Services
Scott Paper Company
P.O. Box 2447
Mobile, Alabama 36601

Dear Mr. Coil:

To date I have received replies from the State, FDA and you concerning the recommendation I prepared in my letter of April 24, 1972. Generally, these Committee members concur with the "E. coli indicator" approach. When I receive comments from the entire Committee I will amend the recommendation, prepare a final draft and mail a copy to you for comments.

May I take this opportunity to thank you for your help on this technical committee.

Sincerely,

Robert F. Schneider
Chief, Biology Branch

RFS/ls

ENVIRONMENTAL PROTECTION AGENCY
Office of Water Programs
Washington, D.C. 20460

May 15, 1972

Mr. Robert F. Schneider
Office of Enforcement
Division of Field Investigations
Building 22, Room 410
Denver Federal Center
Denver, Colorado 80225

Dear Mr. Schneider:

This is in response to your letter of 24 April requesting comments on a proposal to recommend that Federal and State Agencies combine their efforts to support the research needed to develop a rapid, reliable method for enumerating E. coli in estuary waters used for shellfish harvesting. On this issue, I have the following comments:

1. I have the sincere opinion that the Technical Committee is not in a position to recommend the choice of any one indicator system.

2. The matter of choice of indicator system properly should be made only after careful study of the available data and further data gathered in future investigations.

3. In my opinion, further study should not be conducted only on E. coli. To be meaningful, other coliforms, including total coliforms and fecal coliforms should be included in the investigations. Including these others would provide a bridge from past experience and data on the other organisms and, if contraindications for E. coli are found, more supporting information for coliforms or fecal coliforms would become available.

C. B. Kelly
Special Assistant for
Criteria and Standards
Water Supply Programs Division

ENVIRONMENTAL PROTECTION AGENCY

REPLY TO ATTN OF: Chief, State Program Grants Section DATE: May 24, 1972

SUBJECT:

TO: Mr. Robert F. Schneider
Chief, Biology Branch
Denver Center

From your memorandum and attached paper by Clem and Hunt, I presume that the direction for seeking a suitable indicator organism to aid in the sanitary classification of shellfish growing areas lies toward the utilization of the organism, E. Coli.

The course which is recommended represents a progressive step toward our refining the bacteriological spectrum of the general coliform organism group for use in sanitary classification of shellfish growing areas. First, the coliform test was used for classification purposes, then in the early 1960's - and after a lot of research - the "fecal coliform" test was accepted as a supplementary test for classifications. The test, which was widely used to obtain supplementary information, was never accepted as an alternate to the coliform test. Now going to the E. Coli test represents a narrowing of the spectrum.

The proposal in your memorandum suggests that a study be made to determine the suitability of using E. Coli as an indicator organism. This may be a good realistic approach to establish an organism with fecality integrity which will provide a true index of the sanitary quality of shellfish growing waters. There has been, however, a great deal of experience utilizing the fecal coliform group in shellfish work and a lot of research too. First, I suggest that the committee not be hasty in turning away from this accumulated experience and, therefore, recommend that a hard look be taken at the vast amount of F.C. information which has been collected and applied. Secondly, if the committee elects to move forward toward encouraging the development of a rapid and accurate E. Coli test, then such development must move forward with all deliberate speed.

It would be interesting to have C. B. Kelly's and Dr. Monty's comments on the suggested course of action before a firm commitment to move ahead with the E. Coli study is made. It would also be interesting to look into the standards and procedures used by the English, French,

Portuguese, Spaniards, and Japanese in utilizing bacterial indices for classifying shellfish growing areas. While it may not be necessary, I hope we don't get involved in a protracted study - which includes that more study is needed before definite recommendations can be put forth.

Frank J. Silva

State of Alabama
Department of Public Health
State Office Building
Montgomery, Alabama 36104

May 22, 1972

Mr. Robert F. Schaefer
Chief, Mining Branch
Environmental Protection Agency
Office of Enforcement
Bldg. 22, Noon Air, Denver Federal Center
Denver, Colorado 80225

Dear Mr. Schaefer:

I have asked our Director of Laboratories, Mr. Thomas S. Berry, to give us his input and recommendations in regard to proposals to evaluate [illegible] of pollution in Cahaba River Watershed areas.

Yours very truly,

Arthur J. Bedsole
Chief Engineer & Director
Bureau of Environmental Health

AJB/ds

cc: Mr. Thomas S. Berry

State of Alabama
Department of Public Health
State Office Building
Montgomery, Alabama 36104

IRA L. MYERS, M. D.
STATE HEALTH OFFICER

August 7, 1972

Mr. Harvey Stein
Chief Enforcement Officer - Water
Crystal Mall Bldg. 2
Room 1113
Washington, D. C. 20503

Dear Mr. Stein:

I apologize for not advising Mr. Robert P. Schneider, Chairman, Public Hygiene Committee before now of receipt of your letter in regard to an inference zone for pollution control of water carrying wastes.

Probably more time has been done on fecal coliform organisms with regard to water pollution control than on any other area of the field. Many years ago a water-borne typhoid epidemic prompted the analysis of thousands of water and oysters relative to the fact that the B.C. zone is the most logical test to indicate water pollution in public use. Essentially, here with the Public Health service and the army, we have come to agreement with our reasoning and some people still are.

At the state conference the problem arose that we should look further into the possibility of developing a more practical criterion to more effectively evaluate the probability of pathogen presence. This is the charge to the people in recreational waterways.

I do enclose a memorandum to me from Dr. Thomas S. Leahy, Director of our State Laboratory, in which other documents that relate to the subject. Dr. Leahy and others have been familiar with coliform-fecal analyses do not feel that the E. coli test with all of its qualifications is a specific test for E. coli.

I do not know what test one or will be used by regulatory authorities for the control of shellfish growing waters at other sections of the Gulf Coast, but we in Alabama still feel that the F.C. test as we have used it, should be the official standard test.

Mr. Murray Stein

August 7, 1972

Again I apologize for being late with my report to you and Mr. Schneider.

Yours very truly,

Arthur J. Dyck
Chief Engineer & Director
Bureau of Environmental Health

AJD/iw
encl.

cc: Mr. Robert F. Schneider

Dr. Thomas S. Hoey

State of Alabama
Department of Public Health
Bureau of Laboratories
434 Monroe Street
Montgomery, Alabama 36104

IRA L. MYERS, M.D.
STATE HEALTH OFFICER

THOMAS S. HOSTY, PH.D.
DIRECTOR LABORATORIES

August 4, 1972

MEMORANDUM

TO: Mr. Arthur Beck

FROM: Thomas S. Hosty, Ph.D., Director
Bureau of Laboratories

Reproduced from best available copy.

SUBJECT: Mobile Bay

I did not write to Mr. Schneider as I told you I would since I felt that the meeting we had in Virginia would give a satisfactory summation of Alabama's stand. All of the testimony given in Virginia was transcribed and should be readily available to TVA. Many of the representatives of TVA were present at the meeting. I think it is worthy of note that the opposition of the TVA staff was more or taken aback very nicely. I was extremely impressed at the fact that Mr. Schneider was one of the most knowledgeable people in the audience as to the situation that is in Mobile Bay. It is my opinion that the least solid point in all of our testimony was the failure to show that every service [illegible] to a certain degree to our municipal docks to justify fecal coliforms. We were advised before going to Virginia that we should be able to demonstrate what we were saying. Through the years the operation results fine to say because they please - rarely without accurate documentation.

Recent dredging jobs in this area have [illegible] carefully analyzed. Certainly we are not against the dredging of a ferry dock. I made a statement in regard that some [illegible] could not be considered as an L.coli test because [illegible] and we can be opposite to this statement. Dr. Hall very eventually followed the history of the fecal coliform test and pointed out that even the author of the [illegible] did not consider this to be a specific test for L.coli.

A considerable amount of discussion has been made relative to the growing of [illegible] in Mobile Bay. Certainly it is probably [illegible] and the water temperature that are present and a [illegible] of this [illegible] but I find it hard to believe that [illegible] would not also grow under the same conditions. For [illegible] we will ever run test [illegible] that [illegible] to [illegible] give a positive TC test. [illegible] and we are not data would [illegible]

[illegible] probably due to [illegible] results on [illegible]

State of Alabama
Department of Public Health
Bureau of Laboratories
434 Monroe Street
Montgomery, Alabama 36104

IRA L. MYERS, M. D.
STATE HEALTH OFFICER

THOMAS S. HOSTY, PH.D.
DIRECTOR LABORATORIES

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and subsequent tests. It was found later that sewerage from the plant was discharged into paper mill waste with the F.C. responding very nicely.

Mr. Salinger from Maryland presented a very nice study on the relationship of fecal coliform results to polluted, clean and intermediate types of pollution and that study has become part of the record of the Virginia meeting. A copy of Mr. Salinger's report is included with this memo as well as the study on the new Scott paper mill.

I am also enclosing a part of our study of station 119 and it shows rather dramatically not only the ability of the fecal coliform test to detect pollution but coincides quite well with the dropping of salinity. These studies were continued on at least 25 other stations during the course of three years.

May I refer you to the Denver, Colorado meeting of May 19, 1972 with particular reference to item one of your memorandum "The fecal coliform is a valid indicator of fecal pollution in all waters."

I was very interested in the tentative approval of a test developed by the Canadian [illegible] which was entitled "The Rapid Recovery of Small Fecal Coliform Colonies." The article appears to have considerable merit but really we would like Dr. Presnell to read more than one article before he [illegible] Scott and his test on a matter of 25 tests on a limited area. We are at present using a slightly modified test on waters not known I am certain Dr. Presnell would not advocate that this test be approved immediately without extensive studies on a wide variety of waters over the area where these studies were made in the Canadian waters as well as the areas over the entire area. At any rate we will continue our limited studies in this and I am will advise.

For many years the cooperative agreement between states and shellfish people has benefited the whole shellfish program and may I say now in this fifty years of cooperative agreement to be nullified by not calling the shots. Our data over these years proved that FC test could be used in Alabama with a [illegible] of good [illegible] and we would still like to use it as a warning of possible pollution but we must all of the tools to be reviewed sometimes even [illegible] before the [illegible] returned to normal. Certainly our first [illegible] should be [illegible] and [illegible] should be done [illegible] is [illegible] again of the existing test and account to accurate new ones.

APPENDIX E

RAPID RECOVERY OF *ESCHERICHIA COLI*

FROM ESTUARINE WATERS

COPY

ABSTRACTS OF RAPID METHODS FOR THE RECOVERY OF *Escherichia coli*

Three rapid methods for the recovery of *Escherichia coli*, which appear applicable to shellfish and shellfish waters, are abstracted.

METHOD A

Fishbein *et al.* investigating the recovery of *E. coli* from marine products and other foods found that the best recovery was obtained from lauryl sulfate tryptose (LST) broth incubated at 44 C. The method is a fermentation tube technic in which the samples are inoculated directly into LST broth and incubated at 44 C in a water bath. Since 96 percent of the *E. coli* recovered by the method were recovered with 24 hours it is essentially a 24-hour test. Results obtained by the method were comparable to those obtained by the Association of Official Analytical Chemists (AOAC) test. The latter test is much longer, requiring from two to four days.

A complete discussion of the method appears in *Applied Microbiology*, Vol. 15, No. 2, pp 233-238, under the title, "Coliform Behavior in Frozen Foods."

METHOD B

Andrews and Presnell of the Food and Drug Administration, Gulf Coast Technical Services Unit, have developed and evaluated a 24-hour, elevated temperature, tube fermentation, most probable number test. The medium, designated as A-1, contains concentrations of lactose, tryptone, and sodium chloride found in most commercial media. In

addition the medium contains trition X-100, a surfactant, and salicin, a carbohydrate readily utilized by *E. coli*. This method involves the direct inoculation of the sample, in appropriate dilutions, into the medium which is incubated for 24 hours at 44.5 C in a water bath. The method has been compared with the procedure contained in the fourth edition of *APHA Recommended Procedures for the Bacteriological Examination of Sea Water and Shellfish* and the LST elevated temperature (44 C) test described by Fishbein. The APHA recommended method requires a 24-to-48-hour presumptive test at 35 C followed by a 24-hour confirmatory test at 44.5 C. The three tests were compared using samples of freshwater, estuarine water, and a limited number of shellfish samples. Results of the comparative study demonstrated that *E. coli* recovery by A-1 medium at 44.5 C and LST at 44 C were comparable and that both methods recovered more *E. coli* than the APHA recommended procedure. The report pertaining to the development and evaluation of the method is included in this Appendix (pp 72 to 82).

METHOD C

Cabelli and Heffernan have described a rapid, elevated temperature plate count method for assaying the *E. coli* content of seawater and shellfish. The medium used is MacConkey's agar modified by decreasing the bile salts concentration from 0.15 percent to 0.075 percent. The sodium chloride eliminated from the medium is replaced, to varying extents, by the addition of shellfish and seawater samples mixed with the medium. In using the method, known weights of

shellfish meats or known volumes of seawater are mixed with a known volume of the medium and distributed into petri plates. The plates are incubated at 45.5 ± 0.5 C for 24 hours. *E. coli* appear as brick red subsurface colonies. A description of the method is published in *Applied Microbiology*, Vol. 19, No. 2, pp 239-244.

RAPID RECOVERY OF *Escherichia coli* FROM ESTUARINE WATERS

WALLACE H. ANDREWS
MAYNARD W. PRESSELL

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Food and Drug Administration
Bureau of Foods
Office of Food Sanitation
Division of Shellfish Sanitation
Gulf Coast Technical Services Unit
Dauphin Island, Alabama

ABSTRACT

The efficiences of two 24-hr elevated-temperature tests to recover *E. coli* from estuarine water were compared simultaneously with the 72-hr standard methods procedure of the American Public Health Association (APHA). From 1,710 tubes, *E. coli* was recovered 222 times in lauryl tryptose medium incubated at 44 ± 0.2 C for 24 hr, 261 times in an experimental medium incubated at 44.5 ± 0.2 C for 24 hr, and 257 times by the 72-hr APHA method. The number of false positives enumerated was similar in all three tests. The data indicated that *E. coli* in raw seawater could be determined in 24 hr without a significant loss of accuracy.

INTRODUCTION

The need for a rapid, reliable method for determining the bacterial quality of shellfish-growing waters is an important aspect of the continuing search for methods improvement. The standard methods procedure for the examination of water recommended by the American Public Health Association (APHA) requires a maximum 48-hr enrichment period in lactose broth or lauryl sulfate tryptose broth (LST) to obtain optimum recovery of fecal coliforms. Positive tubes are then transferred to EC medium to determine the most probable number (MPN) of fecal coliforms. Gas production with 24 hr in the fermentation vial of a tube of EC medium incubated in a water bath at 44.5 ± 0.2 C is considered a positive reaction indicating an organism of fecal origin. Tubes that do not produce gas indicate that that the organisms are derived from sources other than the tract of a warm-blooded animal (1). Because of the reported interference by non-fecal coliform biotypes, notably Enterobacter aerogenes and Escherichia intermedium types, with the specificity of the EC medium even at temperatures of 44 C (7,8) and the APHA-adopted 44.5 ± 0.2 C (3,4,6), it is suggested that a determination of the E. coli density would give a more accurate estimate of the degree of fecal pollution of a water sample. Working with frozen foods, Fishbein et al. (5) reported that LST incubated directly at 44 C gave E. coli recovery similar to that of the standard methods procedure of the APHA. A preliminary investigation in our laboratory demonstrated that a newly-formulated medium also gave E. coli recovery comparable to

that of the APHA method. The purpose of this study was to compare, simultaneously, the reliability of E. coli recovery from field seawater samples by use of (i) Pinkbein's elevated-temperature test, (ii) an experimental 24-hr elevated-temperature test with the newly formulated medium, and (iii) the 72-hr APHA standard methods procedure.

MATERIALS AND METHODS

Sixty-eight samples of seawater from various sites in Mobile Bay were collected, iced, and transported to the laboratory. All samples were tested within 24 hr. after collection. Five-tube decimal dilutions were performed in phosphate buffered water (pH 7.2) as far as necessary to obtain a determinable MPN of coliforms. Test IA (LST incubated at 35 ± 0.5 C in a cold-in incubator for 24 to 48 hr) and Test IB (positive tubes from Test IA transferred to EC medium and incubated in a water bath at 44.5 ± 0.2 C for 24 hr) together comprise the APHA standard methods procedure for the examination of water (1). In Test II, LST, and in Test III, Medium A-1 (the newly formulated medium) were inoculated and incubated directly in water baths at 44 ± 0.2 C and 44.5 ± 0.2 C, respectively. Medium A-1 included 5 g lactose, 20 g tryptone, and 5 g NaCl found in most of the commercially prepared dehydrated media; and, in addition, 1 ml Triton X-100 (Rohm and Haas Co.)*, a surfactant, and 0.5 g salicin (Difco), a carbohydrate readily utilized by Escherichia species (2), added to 1,000 ml of distilled water.

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* Mention of commercial products does not imply endorsement by the Food and Drug Administration.

All positive tubes from Tests IB, II, and III were streaked on Levine's eosin methylene blue agar and incubated at 35 ± 0.5 C for 24 hr. One representative colony of each type was picked to lactose broth and incubated at 35 C for 24 and 48 hr. All positive tubes (gas formation) were inoculated into 1% tryptone, M.R.-V.P. medium, and Koser citrate medium and subjected to indole, methyl red, Voges-Proskauer, and citrate (IMViC) determinations at 35 ± 0.5 C. All positive lactose broth tubes were also reinoculated into EC medium and incubated at 44.5 ± 0.2 C for 24 to 48 hr to confirm the ability of the pure culture to grow and produce gas at the elevated temperature.

To facilitate a comparison with the data of Pinkerton et al.(5), we decided to use their terminology, with IA as follows: positive tube, a tube that supports lactose with the production of gas within 24 to 48 hr; negative tube, a tube that does not produce gas within 24 to 48 hr; E. coli$^+$, a tube from which E. coli Type I (++ --) or Type II (- + + -) is isolated; false-positive tube (FP), a tube that passes within 24 to 48 hr, but from which E. coli$^+$ is not isolated; false positive (group), a tube that passes within 24 to 48 hr, but from which a coliform organism other than E. coli$^+$ is isolated; false positive (synergistic), a tube that passes within 24 to 48 hr, but from which neither E. coli$^+$ nor any coliform is isolated.

RESULTS AND DISCUSSION

In Table I, the positive tube reactions of the four tests are compared. LST incubated at 35 C (Test IA) produced the largest number of positive

tubes; all the elevated-temperature tests (IB, II, and III) produced considerably fewer positive tubes. LST incubated directly at 44 C (Test II) produced the fewest positive tubes (631), whereas EC medium (TEST IB) and Medium A-1 (Test III) incubated at 44.5 C produced a similar number of positive tubes (665 and 674).

E. coli recovery data are given in Table 2. Not as many E. coli were recovered in Test II as in Tests IB and III. The productivity ratios (column 3) were obtained by dividing the number of tubes from which E. coli[4] was isolated by the total number of gassing tubes. The observed differences in productivity ratios of the three tests were not considered significant.

Data for the recovery of coliforms, exclusive of E. coli Types I and II, are given in Table 3. These data indicate no significant difference in the number of false-positive tubes encountered by the three elevated-temperature tests.

The results of this study are consistent with those of Fishbein et al. (5) who found that LST incubated at 44 C gave E. coli recovery comparable to that obtained by the APHA standard methods procedure. Although with LST incubated at 44 C we did not recover as many coliforms (631 contrasted to 665) and E. coli (222 contrasted to 257) as were recovered with the standard methods procedure, the differences were not significant.

We wish to emphasize that 44 C, rather than 44.5 C, was used as the temperature of direct incubation of the LST in the water bath, inasmuch as this was the temperature used by Fishbein et al. (5). Furthermore, we

Table 1. Positive tube reactions

(1) Test	(2) Number of positive tubes	(3) Percentage of tubes positive[1]
LST, 35 C, presumptive (IA)	1011	59.12
EC, 44.5 C, confirmatory (IB)	665	38.89
LST, 44 C (II)	631	36.91
Medium A-1, 44.5 C (III)	674	39.42

[1] Based on the number of positive tubes divided by the total number of tubes (1,710).

Table 2. Recovery of *E. coli*

(1) Test	(2) *E. coli*$^{+}$ tubes	(3) *E. coli*$^{+}$/total no. of growing tubes[a]
EC, 44.5 C, confirmatory (II)	257	38.65
LST, 44 C (II)	222	35.18
Medium A-1, 44.5 C (III)	261	38.72

[a]Expressed as a percentage.

Table 3. Coliform group recoveries [a]

(1) Test	(2) Total no. FP[b]	(3) No. FP (group)	(4) No. FP (synergistic)	(5) FP tubes/total no. of gassing tubes[c]
EC, 44.5 C, confirmatory (II)	406	385	21	61.06
LST, 44 C (II)	407	389	18	64.50
Medium A-1, 44.5 C (III)	405	380	25	60.09

[a] All coliforms and Escherichia species exclusive of E. coli Types I and II.

[b] FP-false positives; gassing tubes from which E. coli was not recovered.

[c] Expressed as a percentage.

believe the use of 44 C is justified since the recovery of E. coli and the enumeration of false positives by the LST at this temperature were not significantly different from those by the APHA method. With Medium A-1, however, a temperature of 44.5 C was used because this is the most acceptable temperature for the incubation of the fecal coliform group (1). The effects of the direct incubation of LST and Medium A-1 over a range of temperatures will be the subject of a subsequent report. When the overall performance of Medium A-1 was compared with that of the APHA procedure, results by both methods were similar in enumerating coliforms (674 and 665) and E. coli (261 and 257). When the two rapid methods were compared to each other, Medium A-1 recovered more coliforms (674 compared to 631) and E. coli (261 compared to 227) than did LST incubated at 44 C. Both rapid tests merit further investigation because of the savings in time and cost of media, without an accompanying loss in accuracy. It would be particularly significant if a rapid, reliable method could be adopted as a standard methods procedure for determining the bacterial quality of shellfish-growing waters as well as the bacterial content of oysters, clams, and other potentially marketable seafoods.

ACKNOWLEDGMENT

We wish to express our appreciation to Jack Caires and Merrill McPhearson for collecting samples and to Bob Pantley and Clark Jones for their very capable technical assistance.

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